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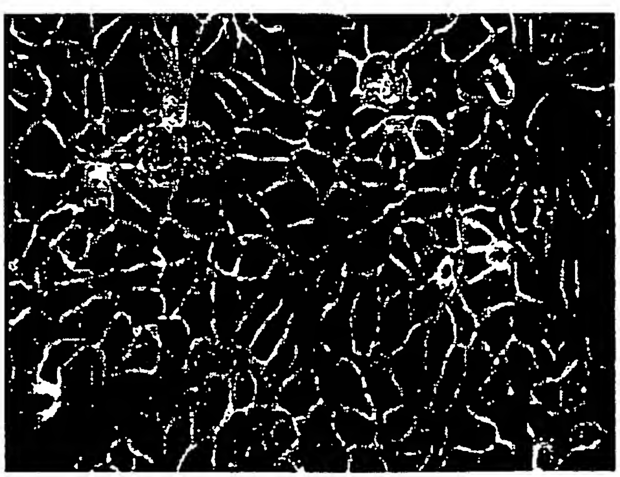

(57) Abstract: An increasingly aged population and better diagnosis has lead to an apparent increase in the prevalence of prostate cancer in men. There is an acute need to better understand the progression of this disease from its locally confined site of initiation to the end stage widely metastatic disease with attendant morbidity and mortality. It has historically been difficult to raise and maintain immortalised prostate cell lines in culture. We have derived a cell line selected from the group consisting of clones ONYCAP 1 and ONYCAP23. The cell lines are characterised as being prostate epithelial in origin.

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New Prostate Cell Lines

Summary

An increasingly aged population and better diagnosis has lead to an apparent increase in the prevalence of prostate cancer in men. There is an acute need to better understand the progression of this disease from its locally confined site of initiation to the end stage widely metastatic disease with attendant morbidity and mortality. It has historically been difficult to raise and maintain immortalised prostate cell lines in culture. For some 15 -20 years the field of in vitro experimentation in prostate cancer has relied upon three cell lines derived from metatstatic sites. More recently several new cell lines have been derived from primary tissue by means of immortalisation with viral oncogene constructs. We have derived a series of immortal cell line clones. The cell lines are characterised as being prostate epithelial in origin and have excellent growth characteristics in combination with unusual expression of markers that make these cell lines valuable for antigen discovery and use as potential vaccines in the treatment of prostate cancer as well as for the purposes of drug screening, genetic analysis of the basis of prostate cancer and other relevant studies.

Field of the Invention

Carcinoma of the prostate (PCA) is the second-most frequent cause of cancer related death in men in the United States (Boring, 1993). The increased incidence of prostate cancer during the last decade has established prostate cancer as the most prevalent of all cancers (Carter and Coffey, 1990). Although prostate cancer is the most common cancer found in United States men, (approximately 200,000 newly diagnosed cases/year), the molecular changes underlying its genesis and progression remain poorly understood (Boring et al., 1993).

An unusual challenge presented by prostate cancer is that most prostate tumors do not represent life threatening conditions. Evidence from autopsies indicate that 11 million American men have prostate cancer (Dbom, 1983). These figures are consistent with prostate carcinoma having a protracted natural history in which relatively few tumors progress to clinical significance during

the lifetime of the patient. If the cancer is well-differentiated, organ-confined and focal when detected, treatment does not extend the life expectancy of older patients.

Unfortunately, the relatively few prostate carcinomas that are progressive in nature are likely to have already metastasized by the time of clinical detection. Survival rates for individuals with metastatic prostate cancer are quite low. Between these two extremes are patients with prostate tumors that will metastasize but have not yet done so. For these patients, surgical removal of their prostates is curative and extends their life expectancy.

Historically there have been few immortal prostate lines which may be cultured in vivo for use in drug screening, antigen discovery or other experimental techniques seeking new therapeutic entities for this disease. Three cell lines have been in widespread experimental use for some 15-20 years namely ; DU145, (Mickey, et al., Cancer Res. 37: 4049-4058, 1977; K.R. Stone, et al., Int. J. Cancer 21: 274-281, 1978); PC-3 , (M.E. Kaighn et al Invest. Urol. 17: 16-23, 1979; Cancer Res. 40: 524-534, 1980) and LnCap (Horoszewicz J.S. et al, Models for Prostate Cancer, 1980, Alan R, Liss Inc, 150 Fifth Avenue, New York NY. NY 10011).

More recently a growing number of groups have developed new cell lines utlising virally derived oncogenes to achieve immortal status in culture. These cell lines include ; TSU-Pr1, (Iizumi T et al. J Urol 1987 Jun;137(6):1304-6, Establishment of a new prostatic carcinoma cell line TSU-Pr1); LuCap23 (Ellis WJ. et al, *Clin Cancer Res* 1996 Jun;2(6):1039-48, Characterization of a novel androgen-sensitive, prostate-specific antigen-producing prostatic carcinoma xenograft: LuCaP 23); P69SV40-T P69-M2182, (Plymate SR. et al *J Clin Endocrinol Metab* 1996 Oct;81(10):3709-16, The effect on the insulin-like growth factor system in human prostate epithelial cells of immortalization and transformation by simian virus-40 T antigen); MDA PCa 2a and MDA PCa 2b (Navone NM. et al., *Clin Cancer Res* 1997 Dec;3(12 Pt 1):2493-500 Establishment of two human prostate cancer cell lines derived from a single bone metastasis) ; 1519-CPTX, 1535-CPTX, 1532-CPTX and 1542-CP$_3$TX (Bright RK.et al., Cancer Res 1997 Mar 1;57(5):995-1002 Generation and genetic characterization of immortal human prostate epithelial cell lines derived from primary cancer specimens) and the ARCAP cell line (Zhau H.Y. et al Androgen-repressed phenotype in human prostate cancer *Proc Natl Acad Sci U S A* 1996 Dec 24;93(26):15152-7)

Prostate cancer in situ and also cell lines from primary and metastatic sites in common with many tumour types have been shown to down regulate their MHC-I expression by a variety of mechanisms (Blades RA et al Urology 1995 Nov;46(5):681-6; discussion 686-7 Loss of HLA class I expression in prostate cancer: implications for immunotherapy). This has implications for immunotherapeutic strategies in vivo and also antigen discovery in vitro both of which benefit significantly from MHC-1 expression. In vivo, lack of MHC-1 expression will disable the direct presentation by tumour cells of T cell epitopes to the T cell receptor on both CD4 and CD8 cells and thereby effectively become invisible to T cell mediated killing. In vitro immortalised cells lacking MHC-1 expression are significantly less effective in a number of experimental uses including T cell mediated lysis assays and peptide elution studies. Therapeutic uses of cell lines in the form of whole cell vaccines also benefit from MHC-1 expression, particularly in allogeneic vaccination strategies. In experimental models of vaccination against tumour challenge "allo" MHC-1 expression in the vaccinating cell line produces the most marked protective effect particularly when the allo MHC-1 resides on the tumour cell carrying relevant tumour associated or tumour specific antigens (Xu W et al Cancer Immunol Immunother 1998 Jan;45(5):217-24).

Specific Field of the Invention

Prostate cancer in most cases remains localised within the prostate itself and does not escape the local confines of the prostate. Thus unless patients are monitored clinically by way of blood PSA level, digital rectal examination, ultrasound or needle biopsy the lesion is not diagnosed. When the tumour does escape from the prostate gland the spread and the favoured metastatic sites are very reproducible. The main sites of deposition are the local lymph nodes and more extensively the bone, in fact very often the first diagnosis of prostate disease is bone pain or non specific fractures of the bone resulting from bone metastatic deposits. The reasons for the preponderance of lymph and bone metastasis may be the local proximity of the lymph nodes and the growth factor rich environment of the bone. There have been relatively few reports of cell lines generated from other metastatic sites, the most notable being the derivation of DU145 from a brain metastasis and the ARCAP cell line derived also from ascitic fluid of a patient with widely disseminated prostate cancer.

The first embodiment of this invention is two cell clones ONYCAP1 and ONYCAP23. The cell lines have been extensively characterised as being prostate epithelial in origin by virtue of cytokeratin staining. The cell lines are further shown to posses significant levels of surface MHC-1 expression in addition to several other immune functional proteins not normally attributed to tumour cell lines, namely MHC-2, ICAM, and CD40 ligand. The two clones show differing morphology and also a differing pattern of gene expression with Onycap23 showing a distinct osteomimetic phenotype compared to Oncap1.

In our application number GB 0008032.5 (01.04.00) we believed that these cells derived from the ascitic fluid but we now know they are derived from PNT-2 cells (see the Examples).

A second embodiment of this invention is the use of any one of the cell lines in the formulation of a vaccine for the treatment of prostate cancer with or without a vaccine adjuvant which may include Il-2, IL-12, interferon gamma, BCG, tetanus toxoid or Mycobacterium Vaccae. The vaccine may be used as an adjuvant therapy in combination with other treatment modalities such as radiotherapy, or surgery or chemotherapy where the vaccine is used to treat or resolve minimal residual disease.

A further aspect of the invention is the use of a combination of the cell lines in the formulation of a vaccine for the treatment of prostate cancer with or without a vaccine adjuvant which may include Il-2, IL-12, interferon gamma, BCG, tetanus toxoid or Mycobacterium Vaccae. The vaccine may be used as an adjuvant therapy in combination with other treatment modalities such as radiotherapy, surgery or chemotherapy where the vaccine is used to treat or resolve minimal residual disease

A further aspect of the invention is the use of a combination of any of the cells with other prostate cell lines available from ATCC, ECACC or other laboratories and cell banks in the formulation of a vaccine for the treatment of prostate cancer with or without a vaccine adjuvant which may include IL-2, IL-12, interferon gamma, BCG, tetanus toxoid, Mycobacterium Vaccae or another suitable adjuvant or immunomodulator known in the art. The vaccine may be used as an adjuvant therapy in combination with other treatment modalities such as radiotherapy, surgery or

chemotherapy where the vaccine is used to treat or resolve minimal residual disease or for the treatment of the disease at any stage with or without co-treatment of various types.

An unexpected finding arising from the analysis of these cell lines is the finding that they possess significant surface levels of MHC-1 which offers the potential to use the cell lines in peptide elution studies to isolate and identify MHC-1 restricted peptides from these cell lines. These cell lines are also unique both in the spontaneous nature of their immortalisation and the highly aggressive manner in which they have developed in vivo. They therefore represent an excellent source of potential antigens relevant for metastatic prostate disease. A further embodiment of this invention is therefore the use of these cell lines to identify MHC-1 restricted peptides and tumour antigens, peptides and proteins arising from these cell lines and vaccines prepared utilising antigens, peptides or DNA vaccines derived from these cell lines.

A further embodiment of this invention are expression libraries derived from the cell lines which can be used in screening experiments to discover tumour associated or specific antigens for use as vaccine or immunotherapies and diagnostics. Expression cloning is now technically simple utilising commercially available kits such as the epitope tagged bacterial expression kits from Invitrogen™ and Roche™ or mammalian expression kits from Statagene™. Once the RNA has been reverse transcribed to cDNA and inserted into a relevant expression system the various clones can be probed with anti-serum from vaccinated or non vaccinated patients using a SEREX type approach. Alternatively mammalian cell expression clones can be used as targets for use with cytotoxic T-cells (CTL) from vaccinated or unvaccinated animals or patients to identify potential T cell antigens

In a further embodiment the cell lines may be used in specific proliferation experiments utilising whole blood to determine the precursor frequency of T cells that recognise antigens derived from the cell lines in both vaccinated and non-vaccinated patients. We have surprisingly found that lysates of these cell lines are good at stimulating T cell proliferation in a high percentage of non-vaccinated patients indicating that there may be many shared antigens between these novel cell lines and early stage tumours in situ. Lysates of the cells may also be used to pulse antigen presenting cells or other cells expressing MHC-1 to enable these cells to be used as potential targets in CTL assays

Further embodiments of this invention relate to use of the cell lines grown in nude mice for drug screening, use of the cell lines in genomic screens for drug target identification and identification of antigens which may be used in diagnostic assays to screen for early phase detection of prostate cancer.

Description of Figures

Figure 1: Morphology of Onycap 1 (x200Figure 1A) and Onycap 23 (x 200 Figure 1B)

Figure 2: FACS Analysis of Cytokeratin Expression by Onycap1 and Oncap23

Figure 3: FACS Analysis of Known Immunological Surface Markers Onycap1 (Figure 3A) and Oncap23 (Figure 23)

Figure 4: Proliferation Response of Early Stage Prostate Cancer Patients to Lysates of Onycap1 and Oncap23

Examples

Isolation of the cell lines

A sample of ascitic fluid (3L) was drawn from a patient with known metastatic prostate disease. The ascitic fluid was centrifuged at 1000 x g for 15 minutes and then resuspended in KSFM media supplemented with 25 g/ml bovine pituitary extract, 5 ng/ml of epidermal growth factor, 2 mM L-glutamine, 10 mM HEPES buffer and 5% foetal calf serum (FCS) (hereinafter called "modified KSFM"). Initial outgrowth was monitored and the decanted media was centrifuged to retain any of the non- adherent cells. Over three to four weeks the spheroids attached and uniform cell populations were observed to grow out onto the T75 flasks. It is believed that at some stage during outgrowth contamination occurred with cells PNT-2 [ECACC Reference Number 95012613] which are prostate epithelial cells immortalised with SV40 Large T antigen.

Cloning out the cell lines

A T175 flask of the ascitic outgrowth was trypsinised and plated out onto 96 well tissue culture plates at varying dilutions calculated to give 1, 10 and 100 cells per well. After a period of 14-21 days growth clones were picked from the 1 cell/well plate where there were less than 20 colonies per plate visible. Identified clones were trypsinised and plated into T25 flasks for expansion, after some 14-21 days growth the clones were further expanded to T75 and thereafter T175 flasks. The expanded clones were trypsinised and reformulated in freezing mixture comprising KSFM containing 10% v/v FCS and 20% v/v DMSO and then stored in 1×10^6 cell aliquots in liquid nitrogen.

Characterisation of the clones' morphology

The various clones were characterised on the basis of morphology initially, and example photographs are depicted in **Figure 1.** A small epithelial morphology is shown in Figure 1A and is representative of clone Onycap1. A second unique morphology shown by clone Oncap23 is shown in figure 1B whereby the cells show dendrite-like processes emanating from a central cellular body similar to the so-called neuroendocrine phenotype (Chung TDK and Spiotto MT 2000 The Prostate 42 p186-195) which can be elicited by growth in IL6 containing medium.

Characterisation of the Clones as being of Prostate Epithelial Origin

Onycap1 and Oncap23 clones have been analysed by flow cytometry using anti-cytokeratin 8, anti-cytokeratin 18 and desmin antibodies. Cytokeratin 8 and 18 are characteristic and considered true markers of prostatic epithelia. **Figure 2** shows examples of the flow cytometry analysis of the clones with CY8, CY18 and desmin antibodies compared with isotype control. In all cases the clones stained for these characteristic prostate epithelial markers proving their prostatic origin consistent with the medical condition of the patient

Surface marker analysis of the clones

Selected clones were chosen for surface marker analysis using commercially available antibodies. Analysis was performed to detect the following proteins: MHC-I, MHC-II, CD40, CD154, CD69, CD80 and CD86. **Figure 3** shows representative flow cytometry data for Onycap1 and Oncap23. Unusuallly for prostate metastatic cell lines these clones show siginificant levels of MHC-I along with several other important markers which are important in cell based tumour immunotherapeutics, such as CD86 and CD40.

The presence of siginficant MHC-I levels is important since its cell surface presence will be important in eliciting an allogenic response when these cells are used as an whole cell allogeneic vaccine. There is also the possibility that with surface MHC-I expression these cells may also present directly to T cells, small peptide antigens restricted by the MPA clones MHC-I. In addition once the T cell receptor is engaged the presence of co-stimulatory molecules on the MPA clones may also elicit a significant proliferative response since both T receptor and co-stimulatory signals are present on the MPA cells.

cDNA production and PCR analysis of the clones

Characterisation of the expression profile of Onycap1 and Oncap23 clones was performed by rtPCR analysis with a small number of PCR primers. The expression profile of several well known prostate proteins and antigens in addition to known markers of invasion and metastasis was undertaken to make a preliminary evaluation of these clones to compare them to other known prostate cells. Clones were grown in T75 flasks in KSFM media supplemented with 25 g/ml bovine pituitary extract, 5 ng/ml of epidermal growth factor, 2 mM L-glutamine, 10 mM HEPES buffer and 5% foetal calf serum (FCS).

Cell culture

Cell lines ONYCAP-1 and ONYCAP-23, clones were grown in T75 flasks in modified KSFM. Cells were harvested by trypsinisation from the surface of the plastic and washed in Hanks balanced salt solution before RNA extraction.

RNA extraction

A double extraction was performed using TRI REAGENT (Sigma #T9424). The reagent was added directly to the washed cell pellets and samples were allowed to stand for 5 minutes before the addition of chloroform. Samples were vortexed and allowed to stand for a further 10 minutes at room temperature then centrifuged at 12,000 x g for 15 minutes.

The upper aqueous phase was transferred to a fresh tube, another aliquot of TRI REAGENT added and the above steps were repeated for the second stage extraction. The aqueous phase was again transferred to a fresh tube and the RNA precipitated with isopropanol. RNA pellets were washed with 75% ethanol, dried and re-suspended in TE buffer.

DNase treatment

An aliquot of each RNA sample was treated with Deoxyribonuclease I (Life Technologies #18068-015) to ensure there was no contamination with genomic DNA. Reactions were incubated for 15 minutes at room temperature then the DNase was inactivated by the addition of 25mM EDTA and heating to 65°C for 10 minutes.

Reverse Transcription

Reverse transcription was performed using the 1st strand cDNA synthesis kit for RT-PCR (AMV) from Boehringer Mannheim (#1 483 188). Reactions were incubated at 25°C for 10 minutes then at 42°C for 1 hour. The AMV enzyme was denatured by heating to 99°C for 5 minutes then the reaction was cooled to 4°C.

Results

PCR primers listed were selected from the literature to cover known prostate proteins, antigens and markers of invasion and metastasis. The cDNA extracted from the clones was probed with the

primers and products of the reaction run out on high resolution agarose gels to check for products of the expected sizes. The results are tabulated in **Table 1** for clones Onycap1 and Oncap23.

cDNA production and DNA array analysis of the clones

Methodology

Cell culture

Cell line Onycap1 and Oncap23 were seeded at 1 x 10^6 cells into T175 culture flasks. Cultures were maintained for 4-5 days in modified KSFM. Cells were harvested by trypsinisation from the surface of the plastic and washed in Hanks balanced salt solution before RNA extraction.

RNA extraction

A double extraction was performed using TRI REAGENT (Sigma #T9424). The reagent was added directly to the washed cell pellets and samples were allowed to stand for 5 minutes before the addition of chloroform. Samples were vortexed and allowed to stand for a further 10 minutes at room temperature then centrifuged at 12,000 x g for 15 minutes.

The upper aqueous phase was transferred to a fresh tube, another aliquot of TRI REAGENT added and the above steps were repeated for the second stage extraction. The aqueous phase was again transferred to a fresh tube and the RNA precipitated with isopropanol. RNA pellets were washed with 75% ethanol, dried and re-suspended in TE buffer.

DNase treatment

An aliquot of each RNA sample was treated with Deoxyribonuclease I (Life Technologies #18068-015) to ensure there was no contamination with genomic DNA. Reactions were incubated for 15 minutes at room temperature then the DNase inactivated by the addition of 25mM EDTA and heating to 65°C for 10 minutes.

DNA Array Probing

Labelled cDNA was used to probe a Clontech AtlasTM array (human cancer array II) following the manufacturers protocol for hybridisation and washing. Images were recorded utilising a phosphor imager and recorded files analysed with the Clontech Atlas Image TM software.

Results

Expressed genes are tabulated in **Table 2** and show the clones Onycap1 and Oncap23 to share many common expressed genes but also that they possess unique expressed products as predicted by their very different morphology

Human T Cell Proliferative Response to Lysates of Onycap1and Oncap23

Methodology

Preparation of MPA clone lysates for T cell proliferation studies.

Clones were grown in T75 flasks in modified KSFM. Once the cells have reached confluency they were harvested by trypsinisation and washed in Hanks balanced salt solution and then pelleted by centrifugation.

The pelleted cell mass was taken through four freeze thaw cycles in a minimal volume of Hanks balanced salt solution at a cell concentration of $2x10^6$ cells/ml. The resulting cell supernatent was aliquoted into 25ul aliquots and stored at -70°C until required.

We performed a proliferation assay on T-cells from early stage prostate cancer patients following stimulation with lysates of the prostate cell lines, to determine if at an early stage of the disease T-cell populations harbored a reactivity to antigens derived from the Onycap1 and Oncap23 cell clones. Whole blood was extracted at each visit to the clinic and used in a BrdU (bromodeoxyuridine) based proliferation assay as described below:

Patient BrdU proliferation method

Reagents	*Catalogue #*	*Supplier*
RPMI medium		Life Technologies, Paisley Scotland.
BrdU		Sigma Chemical Co, Poole, Dorset.
PharMlyse	35221E	Pharmingen, Oxford UK
Cytofix/Cytoperm	2090KZ	"
Perm/Wash buffer (x10)	2091KZ	"
FITC Anti-BrdU/Dnase	340649	Becton Dickinson
PerCP Anti-CD3	347344	"
Pe Anti-CD4	30155X	Pharmingen
Pe Anti-CD8	30325X	"
FITC mu-IgG1	349041	Becton Dickinson
PerCP IgG1	349044	"
PE IgG1	340013	"

Method

1) Dilute 1 ml blood with 9 ml RPMI + 2mM L-glutamine + penicillin/streptomycin antibiotics + 50μM 2-mercaptoethanol. Do not add serum. Leave overnight at 37°C
2) On following morning, aliquot 450μl of diluted blood into wells of a 48-well plate and add 50μl of stimulator lysate. The lysate is made by freeze-thawing tumour cells ($2x10^6$ cell equivalents/ml) x3 in liquid nitrogen and then storing aliquots frozen until required.
3) Culture cells at 37°C for 5 days
4) On the evening of day 5 add 50μl BrdU @ 30μg/ml
5) Aliquot 100μl of each sample into a 96-well round-bottomed plate.
6) Spin plate and discard supernatant

7) Lyse red cells using 100µl PharMlyse™ for 5minutes at room temperature
8) Wash x2 with 50µl of Cytofix™
9) Spin and remove supernatant by flicking
10) Permeabilise with 100µl Perm/Wash™ for 10mins at RT
11) Add 30µl of antibody mix comprising antibodies at correct dilution made up to volume with Perm/Wash™
12) Incubate for 30 mins in the dark at room temperature.
13) Wash x1 and resuspend in 100µl 2% paraformaldehyde
14) Add this to 400µl FACSFlow™ in cluster tubes ready for analysis
15) Analyse on FACScan™, storing 3000 gated CD3 events.

6-well plate for stimulation

	Nil	Con	15	Ln	D	Pn
PBL 1						
PBL 2						
PBL 3						
PBL 4						
PBL 5						
PBL 6						

96-well plate for antibody staining

PBL 1		PBL 2		PBL 3		PBL 4		PBL 5		PBL 6	
Nil A	15 D	Nil A	15 D	Nil A	15 D	Nil A	15 D	Nil A	15 D	Nil A	15 D
Nil D	15 E	Nil D	15 E	Nil D	15 E	Nil D	15 E	Nil D	15 E	Nil D	15 E
Nil E	Ln D	Nil E	Ln D	Nil E	Ln D	Nil E	Ln D	Nil E	Ln D	Nil E	Ln D
Con D	Ln E	Con D	Ln E	Con D	Ln E	Con D	Ln E	Con D	Ln E	Con D	Ln E
Con E	Du D	Con E	Du D	Con E	Du D	Con E	Du D	Con E	Du D	Con E	Du D
	Du E		Du E		Du E		Du E		Du E		Du E
	Pn D		Pn D		Pn D		Pn D		Pn D		Pn D
	Pn E		Pn E		Pn E		Pn E		Pn E		Pn E

Legend:

A: FITC mu-IgG1 (5µl) + PE IgG1 (5µl) + PerCP IgG1 (5µl) + 15µl Perm/Wash™

D: FITC Anti-BrdU/Dnase (5µl) + Pe Anti-CD4 (5µl) + PerCP anti-CD3 (5µl) + 15µl Perm/Wash™

E: FITC Anti-BrdU/Dnase (5µl) + Pe Anti-CD8 (5µl) + PerCP Anti-CD3 (5µl) + 15µl Perm/Wash™

15: NIH1542-CP3TX (an immortalised line derived from primary prostate cancers by Dr. Suzanne Topalian at the NIH)

Ln: LnCap (ATCC Number: CRL-1740)

D: Du145 (ATCC Number: HTB-81)

Pn: Pnt2 (ECACC Ref No: 95012613)

Con: ConA lectin (positive control)

Nil: No stimulation

Results

The results for a series of proliferation assays are shown in **Figure 4** where a proliferation index for either CD4 or CD8 positive T-cells are plotted against Onycap1 and Onycap 23 cell lysates, the proliferation index being derived by dividing the percentage of T-cells proliferating by the no-lysate control. A proliferation index above 1 indicates significant T cell proliferation in response to the cell lysate used to stimulate.

Results are shown for six early stage prostate patient sera 1,2,3,4,5 and 6 each of which was stimulated with Onycap1 or Oncap23 cell lysate. The results show that the Onycap cell lines posses antigens which differentially stimulate T cells from a variety of patients. Its is unlikely that a response is due to an mixed lymphocyte reaction since both cell lines posses the same haplotype and yet for each of the patients sera there are examples of one cell line stimulating a response where the other cell line does not.

Table 1 PCR Expression Analysis on Onycap1 and Oncap23

Prostate Antigen	Onycap1	Oncap23
PSA	-	-
PAP	-	-
PSM	-	-
Androgen recptr.	-	-
EGF receptor	-	-
IGF-II	-	-
HGF receptor	+	+
uPA	+	+
PCTA-1	+	+
PSCA	+	+
GRP receptor	-	+
uPA receptor	+	+
Hyaluronic acid receptor	-	-
MMP-9	-	-
Vimentin	+	+
PAGE-1	-	-
E-cadherin	-	-
TGFα	+	+
KAI1	-	+
Heparanase	+	+

Table 2 Gene Expression Analysis for Onycap1 and Oncap23

Spot Intensity

Onycap1	Oncap23	Gene product
2748	10332	CD81 antigen; 26-kDa cell surface protein TAPA-1
2260	9368	leukemia virus receptor 1 (GLVR1)
0	7652	lysosome-associated membrane glycoprotein 2 precursor (LAMP2); CD107B antigen
0	5184	lysosome membrane protein II (LIMP II);
0	12796	annexin V; lipocortin V; endonexin II;
0	5688	LGALS3, MAC2 (Galectin-3, MAC-2 antigen,
0	6528	lymphocyte function-associated antigen 3 precursor (LFA3); CD58 antigen
0	28900	neprilysin; neutral endopeptidase (NEP); enkephalinase (EPN);
0	7264	zinc finger protein
248	2676	5'-TG-3' INTERACTING FACTOR (HOMEOBOX PROTEIN TGIF)
0	5032	HOMEOBOX PROTEIN SIX1
2504	4888	SOX-9 PROTEIN
1440	3128	ZINC FINGER PROTEIN SLUG
0	6052	ENX-1 PUTATIVE TRANSCRIPTIONAL REGULATOR
804	7100	LIM AND SH3 DOMAIN PROTEIN LASP-1 (MLN 50)
388	4708	MEL-18 RING-FINGER PROTEIN
8716	17132	NAT1 TRANSLATION REPRESSOR
0	6312	SAP18 (Sin3 associated polypeptide P18)
0	6984	preliminary PRT 640 AA RNA polymerase II elongation factor ELL2
1932	6228	HLA-B-associated transcript 2; large proline-rich protein BAT2
11008	2132	complement 3 (C3)
2076	8868	LANP CEREBELLAR LEUCINE RICH ACIDIC NUCLEAR PROTEIN
184	3164	phospholipid transfer protein precursor (PTLP); lipid transfer protein II
3016	12244	dek protein
0	5452	CYSTEINE-RICH PROTEIN 2 (CRP2) (ESP1 PROTEIN)
3748	8480	RNA-binding protein EWS
6200	15836	RNA-binding protein fus/tls
12996	35868	nucleolar phosphoprotein B23; nucleophosmin (NPM); numatrin
4532	12992	platelet-derived growth factor (PDGF) receptor beta-like tumor suppressor (PRLTS)
1680	5664	retinoic acid receptor alpha
1744	15192	ras-related protein R-ras2; ras-like protein TC21; teratocarcinoma oncogene
0	5676	clathrin assembly protein lymphoid myeloid leukemia (CALM)
27752	40916	MT1H (Methallothionein-IH, Metallothinein-0, MT-0) + Metallothionein isoform 1L (MT-1L)
9124	26864	voltage-dependent anion-selective channel protein 1 (VDAC1);
244	6964	ras-related protein RAB-5C
0	5584	vesicle-membrane fusion protein SNAP23A

3976	16024	annexin IV (ANX4); lipocortin I; calpactin II; chromobindin 9; phospholipase A2 inhibitory protein
13640	34420	annexin I (ANX1)
11428	27236	annexin II (ANX2); lipocortin II; calpactin I heavy subunit; chromobindin 8; protein I;
0	7944	ER lumen protein retaining receptor 2; KDEL receptor 2; ERD 22
0	5136	protein SEC23 homolog isoform A (SEC23A)
2896	11292	coatomer beta' subunit; beta'-coat protein; beta'-COP; p102
0	5472	coatomer delta subunit; delta-coat protein; delta-COP; archain (ARCN1)
0	6448	cation-dependent mannose-6-phosphate receptor precursor
0	7516	syntaxin 7 (STX7)
3464	8192	ras-related protein RAB-11B; YPT3
5740	9960	ras-related protein RAB-7
3852	14512	ras-related protein RAB-1A; YPT1-related protein
640	4004	calcium-binding protein p22; calcium-binding protein CHP
0	9184	clathrin coat assembly protein AP17; plasma membrane adaptor AP-2 17-kDa protein;
8496	19328	clathrin coat assembly protein AP50; plasma membrane adaptor AP-2 50-kDa protein;
0	5908	cytosolic acetoacetyl-coenzyme A thiolase
312	7616	beta-D-galactosidase precursor; lactase; acid beta-galactosidase; GLB1
1212	7572	6-PHOSPHOFRUCTOKINASE,
3492	7320	pyruvate kinase M2 isozyme (PKM2)
708	6620	succinyl-CoA:3-ketoacid-coenzyme A transferase
240	5856	alcohol dehydrogenase 5 chi polypeptide
0	5188	NADH-cytochrome B5 reductase
0	7152	mitochondrial enoyl-CoA hydratase short subunit 1
0	5356	3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; HMGCR)
0	5212	lysosomal acid lipase/cholesteryl ester hydrolase precursor (LAL);
0	8096	phosphatidylethanolamine-binding protein (PBP); neuropolypeptide H3
0	6420	annexin III (ANX3); lipocortin III; placental anticoagulant protein III) (PAP-III);
432	5024	farnesyl pyrophosphate synthetase
448	5276	H105E3 protein
0	5084	dihydrofolate reductase (DHFR)
1976	8296	thymidylate synthase (TYMS; TS)
324	7856	glutamate dehydrogenase 1 precursor (GDH; GLUD1)
2580	5504	very-long-chain-specific acyl-CoA dehydrogenase precursor (VLCAD)
24144	34792	ferritin heavy chain (FTH1); FTHL6
44632	5996	placental type 1 alkaline phosphatase precursor (PLAP-1)
3732	8760	homolog of yeast D2-isopentenylpyrophosphate isomerase (IPP isomerase)
364	6132	ornithine decarboxylase (ODC1)
14304	24032	protein disulfide isomerase
0	5416	cyclophilin 3 protein (CYP3);
14884	31528	60S ribosomal protein L22 (RPL22);

0	8068	TIA-1 related protein; nucleolysin TIAR
0	10204	astrocytic phosphoprotein PEA-15
0	7020	cyr61 protein precursor; GIG1 protein; insulin-like growth factor-binding protein 10 (IGFBP10)
0	5668	granulins precursor (GRN); acrogranin
7288	16696	natriuretic peptide precursor B
932	3548	SEMAPHORIN V
660	8248	signal transducing adaptor molecule (STAM)
2096	4344	beta-adaptin 1; plasma membrane adaptor HA2/AP2 adaptin beta subunit;
128	5132	RIBOSOMAL PROTEIN S6 KINASE (EC 2.7.1.-)(S6K) (P70-S6K).
2796	13364	casein kinase I alpha isoform (CKI-alpha); CK1; CSNK1A
11716	21668	casein kinase I delta isoform (CKI-delta); CSNK1D
812	6076	casein kinase II beta subunit (CK II; CSNK2B; CK2N); phosvitin
2204	7884	MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)
0	5592	protein phosphatase PP2A 65-kDa regulatory subunit alpha isoform;
0	5292	protein phosphatase PP2A 55-kDa regulatory subunit neuronal isoform;
420	5744	dual-specificity protein phosphatase 5; dual-specificity protein phosphatase HVH3
4328	10112	dual-specificity protein phosphatase 7; dual-specificity protein phosphatase PYST2
1484	5124	serine/threonine protein phosphatase 2B catalytic subunit alpha isoform;
972	7260	serine/threonine protein phosphatase 5 (PP5); protein phosphatase T (PPT)
0	6016	protein-tyrosine phosphatase 1B (PTP-1B)
1584	6508	protein-tyrosine phosphatase G1 (PTP-G1)
6480	14912	serine/threonine protein phosphatase PP1-alpha 1 catalytic subunit (PP-1A)
952	6852	guanine nucleotide-binding protein G(K) alpha 3 subunit (GNA3)
4104	12748	GUANINE NUCLEOTIDE-BINDING PROTEIN G(I),
0	8304	ras-related protein RAP-1B; GTP-binding protein SMG p21B
0	6180	calpactin I light chain
8284	15236	ras GTPase-activating-like protein IQGAP1; p19; KIAA0051
856	6416	calmodulin
1556	9264	calmodulin
440	4164	ANNEXIN XI (CALCYCLIN-ASSOCIATED ANNEXIN 50) (CAP-50) (56 KD AUTOANTIGEN).
2756	8752	calgizzarin; S100C protein; MLN70
3768	11592	sorcin 22-kDa protein (SRI); CP-22
472	6296	calcium signal-modulating cyclophilin ligand (CAML)
5352	16056	14-3-3 protein beta/alpha; protein kinase C inhibitor protein-1 (KCIP-1); protein 1054
9088	20912	14-3-3 PROTEIN EPSILON (MITOCHONDRIAL IMPORT STIMULATION FACTOR L SUBUNIT)
4576	11468	14-3-3 PROTEIN ZETA/DELTA (PROTEIN KINASE C INHIBITOR PROTEIN-1) (KCIP-1)
1688	8056	phosphatidylinositol transfer protein alpha isoform (PI-TP-alpha;
12604	26120	guanine nucleotide-binding protein beta subunit-like protein 12;
532	3800	lysosomal protective protein precursor; cathepsin A; carboxypeptidase C; PPGB

9808	23748	calcium-dependent protease small (regulatory) subunit; calpain;
1036	8220	HUNTINGTIN INTERACTING PROTEIN (HIP2)
0	5844	lipoprotein-associated coagulation inhibitor
504	4120	protein-tyrosine phosphatase kappa precursor (R-PTP-kappa; PTPRK; PTPK)
4740	14824	PUTATIVE RECEPTOR PROTEIN (PM1)
40	3116	orphan receptor TR4
168	2980	dynactin 150-kDa isoform; 150-kDa dynein-associated polypeptide (DAP-150;
13744	33716	COFILIN
4044	12584	alpha-actinin 1 cytoskeletal isoform; F-actin cross linking protein
852	5288	drebrin E
1684	11556	major prion protein precursor (PRP); PRP27-30; PRP33-35C; ASCR
820	4628	DXS6673E protein; X-linked mental retardation candidate gene

ecacc
European Collection of Cell Cultures

Centre for Applied Microbiology and Research
&
European Collection of Cell Cultures

This document certifies that Cell Culture
(Deposit Ref. 00032801) has been accepted as a *patent deposit*,
in accordance with
The Budapest Treaty of 1977,
with the European Collection of Cell Cultures on 28TH March 2000

PJ Packer

Dr P J Packer
Quality Manager, ECACC

CAMR
Today's Research
Tomorrow's Health

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No. FS32819

ecacc
European Collection of Cell Cultures

Centre for Applied Microbiology and Research
&
European Collection of Cell Cultures

This document certifies that Cell Culture
(Deposit Ref. 00032802) has been accepted as a patent deposit,
in accordance with
The Budapest Treaty of 1977,
with the European Collection of Cell Cultures on 28TH March 2000

Dr P J Packer
Quality Manager, ECACC

CAMR
Today's Research
Tomorrow's Health

No. FS 33819

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Claims

1. A cell line selected from the group consisting of clones ONYCAP1 and ONYCAP23 which are deposited as Budapest Treaty patent deposit at ECACC on 28th March 2000 under Accession numbers 00032802 and 00032801 respectively, and which cell lines are characterised as being prostate epithelial in origin by virtue of cytokeratin staining.

2. Use of any one of the cell lines of claim 1 in the formulation of a vaccine for the treatment of prostate cancer with or without vaccine adjuvant which may include Il-2, IL-12, interferon gamma, BCG, tetanus toxoid or Mycobacterium Vaccae.

3. Use of a combination of the cell lines of claim 1 in the formulation of a vaccine for the treatment of prostate cancer with or without a vaccine adjuvant which may include Il-2, IL-21, interferon gamma, BCG, tetanus toxoid or Mycobacterium Vaccae.

4. Use of a combination of any of the cell lines of claim 1 with other prostate cell lines available from ATCC, ECACC or other laboratories and cell banks in the formulation of a vaccine for the treatment of prostate cancer with or without a vaccine adjuvant which may include IL-2, IL-12, interferon gamma, BCG, tetanus toxoid, Mycobacterium Vaccae or another suitable adjuvant or immunomodulator known in the art.

5. A vaccine including or consisting of cells from one or both cell lines of claim 1, optionally together with an adjuvant.

6. An Expression Library derived from one or both cell lines of claim 1 which can be used in screening experiments to discover tumour associated or specific antigens for use as vaccine or immunotherapies and diagnostics.

7. Use of one or both of the cells of claim 1 in specific proliferation experiments utilising whole blood to determine the precursor frequency of T cells that recognise antigens derived from the cell lines in both vaccinated and non-vaccinated patients.

8. Use of the cell lines of claim 1 grown in nude mice for drug screening.

9. Use of the cell lines of claim 1 in genomic screens for drug target identification and identification of antigens which may be used in diagnostic assays to screen for early phase detection of prostate cancer.

Figure 1

Figure 1A

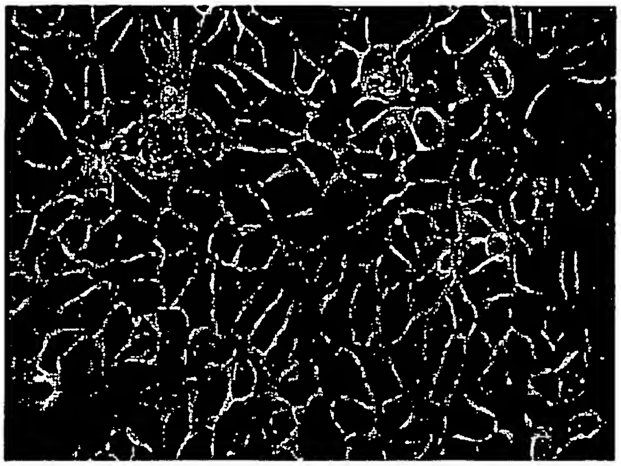


Figure 1B

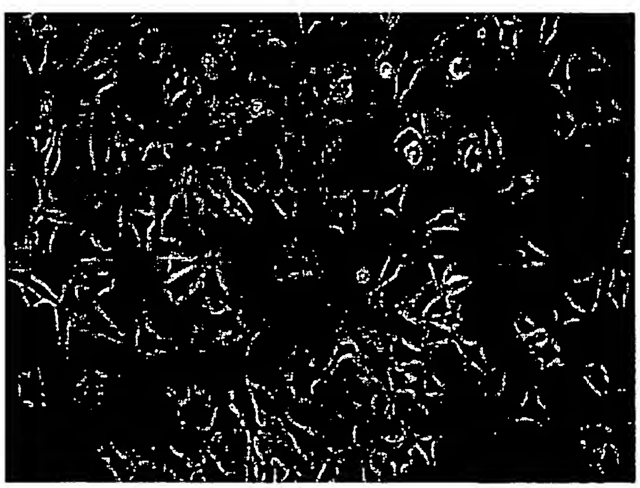
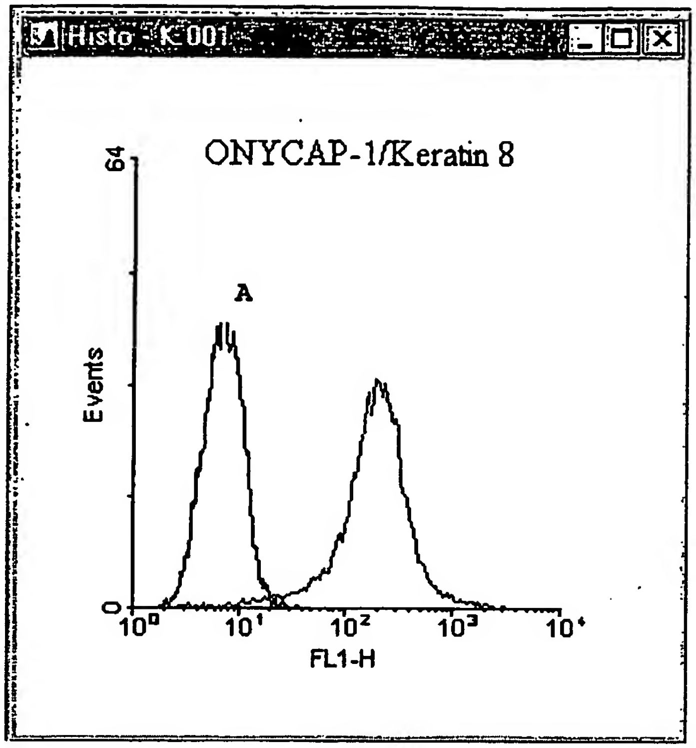
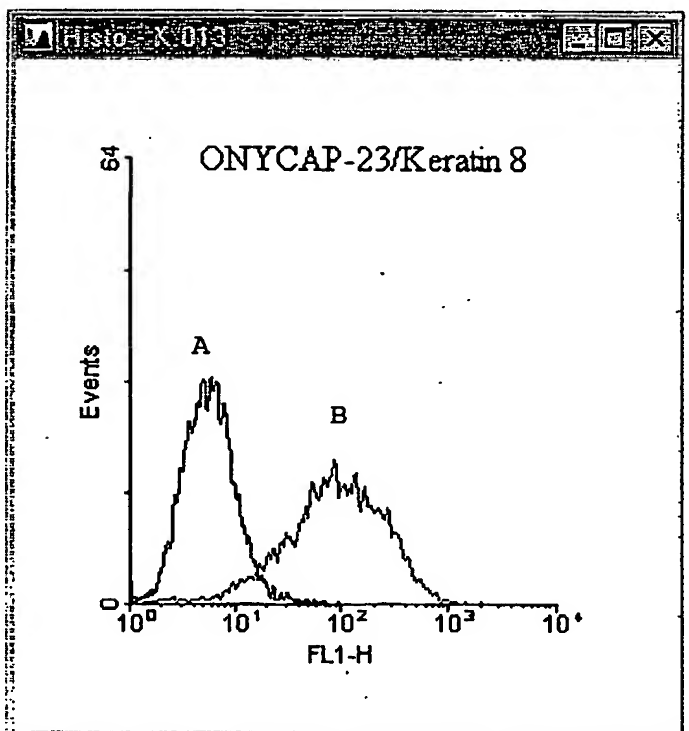


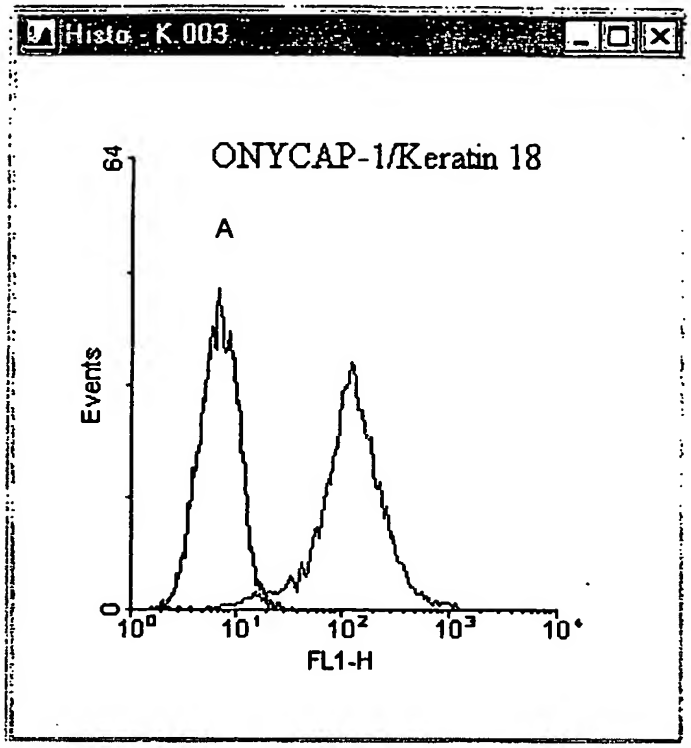
Figure 2a

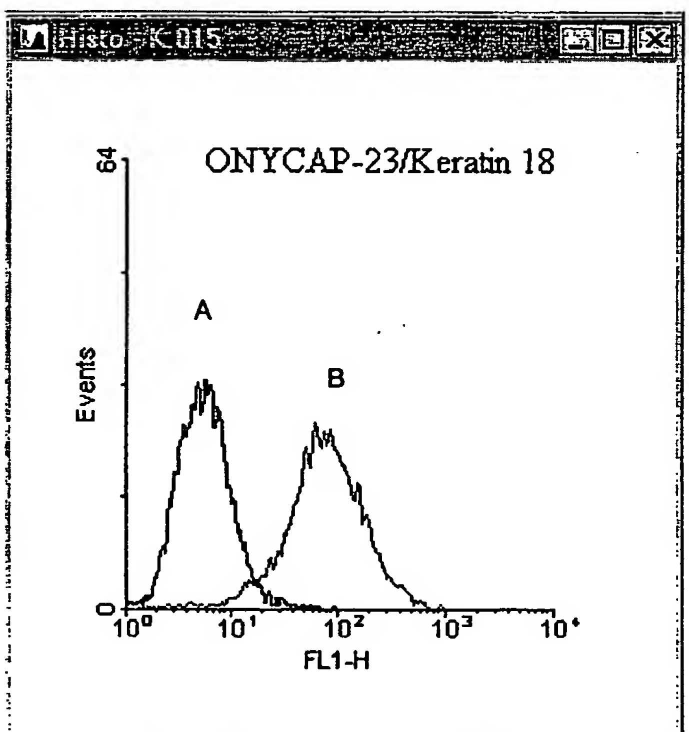




A = Isotype control plot
B = Specific antibody staining

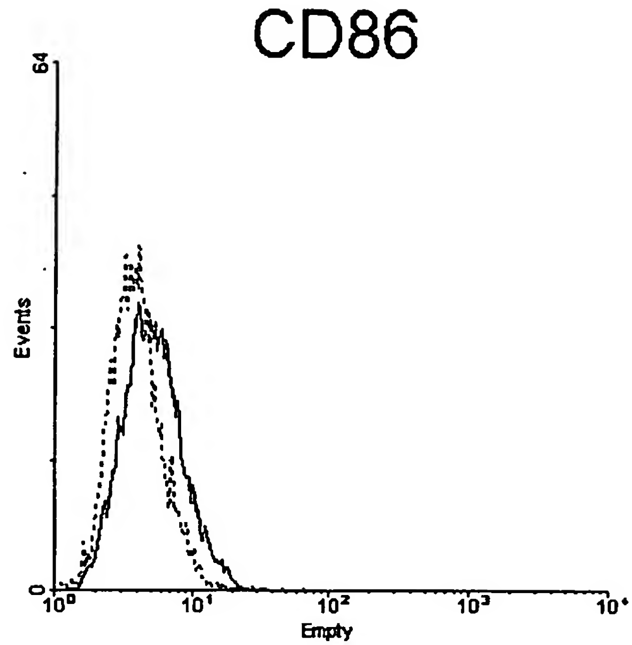
Figure 2b

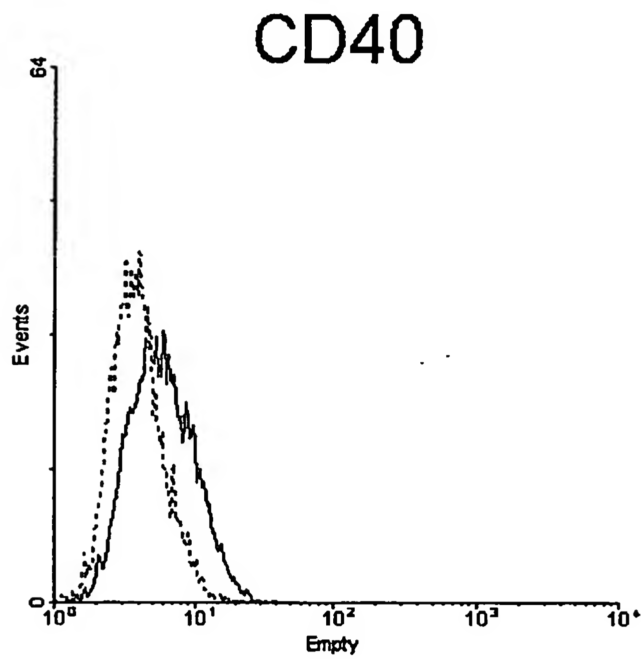




A = Isotype control plot
B = Specific antibody staining

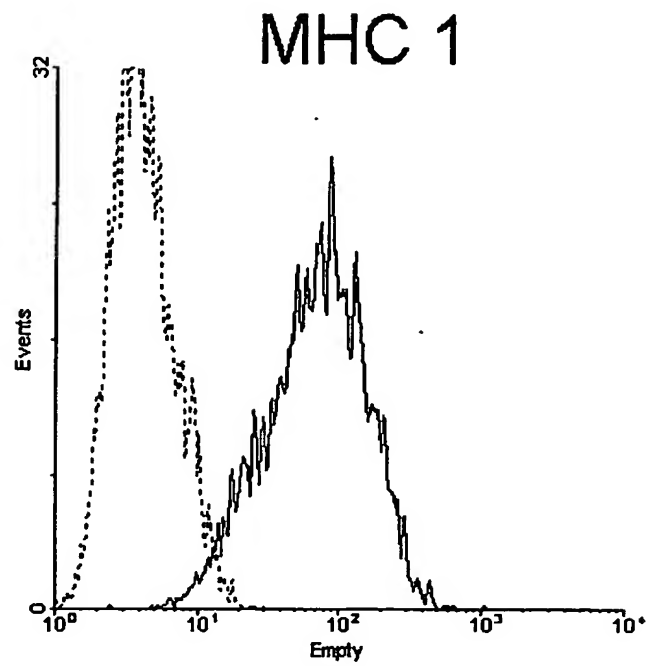
Figure 3Ai: Onycap1-

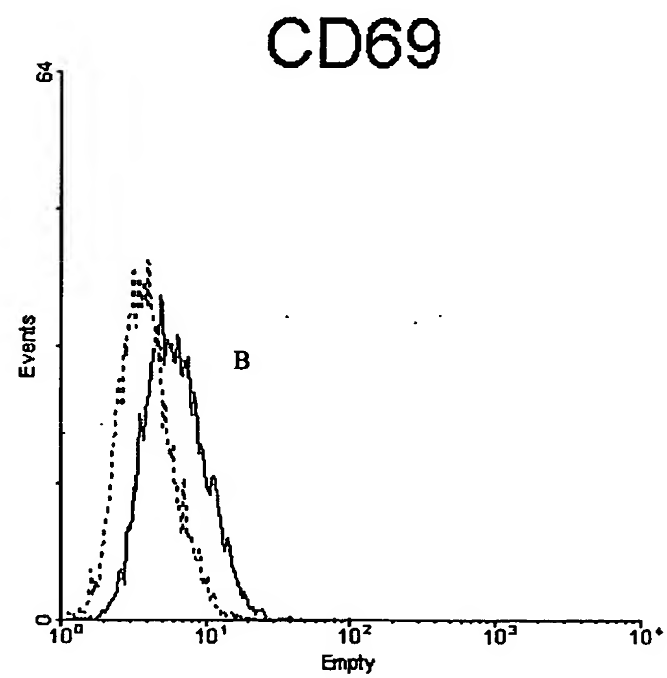




A = Isotype control plot
B = Specific antibody staining

Figure 3Aii

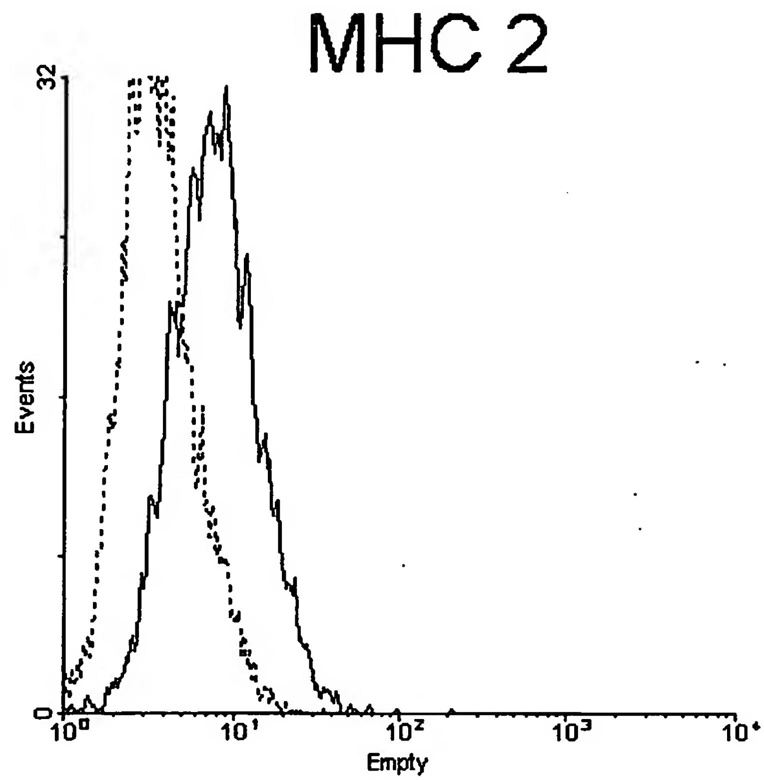




A = Isotype control plot

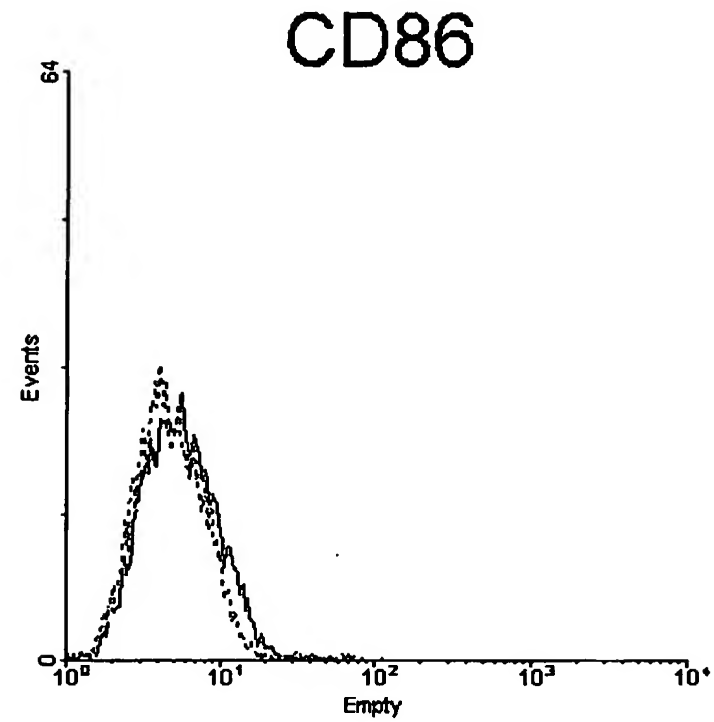
B = Specific antibody staining

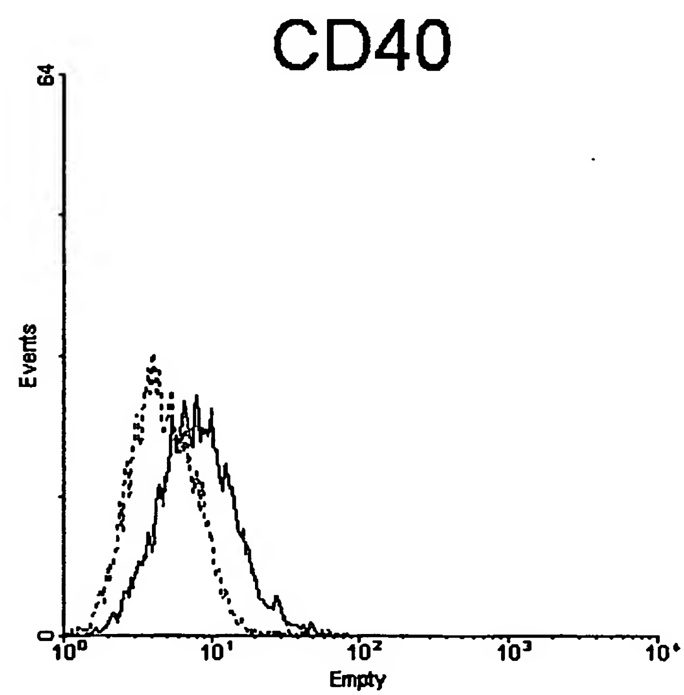
Figure 3Aiii



A = Isotype control plot
B = Specific antibody staining

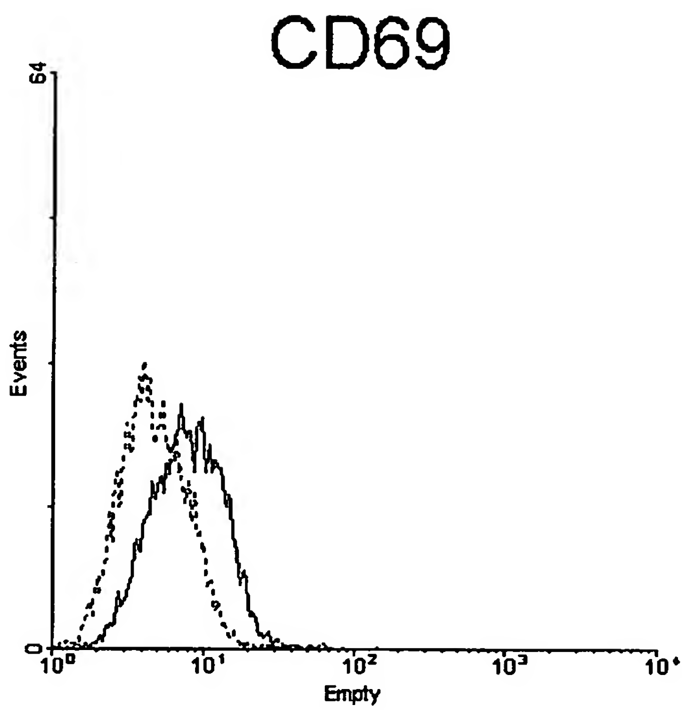
Figure 3Bi:- Onycap 23

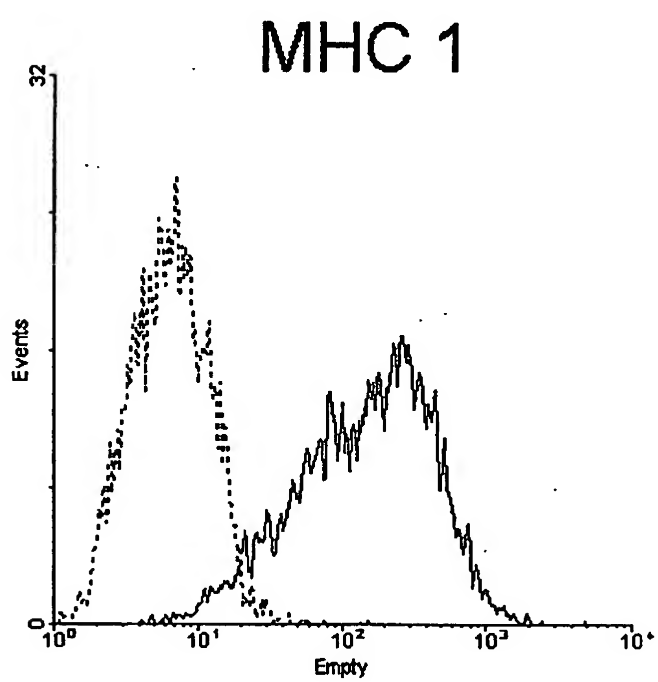




A = Isotype control plot
B = Specific antibody staining

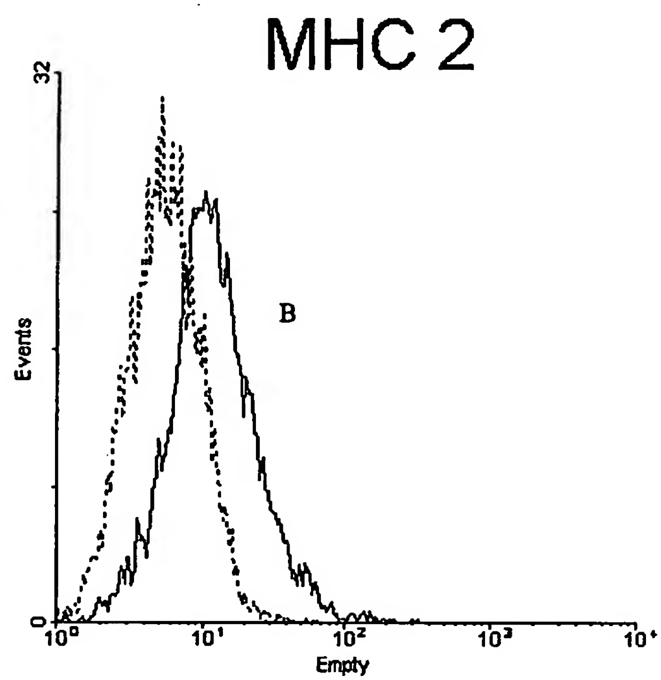
Figure 3Bii





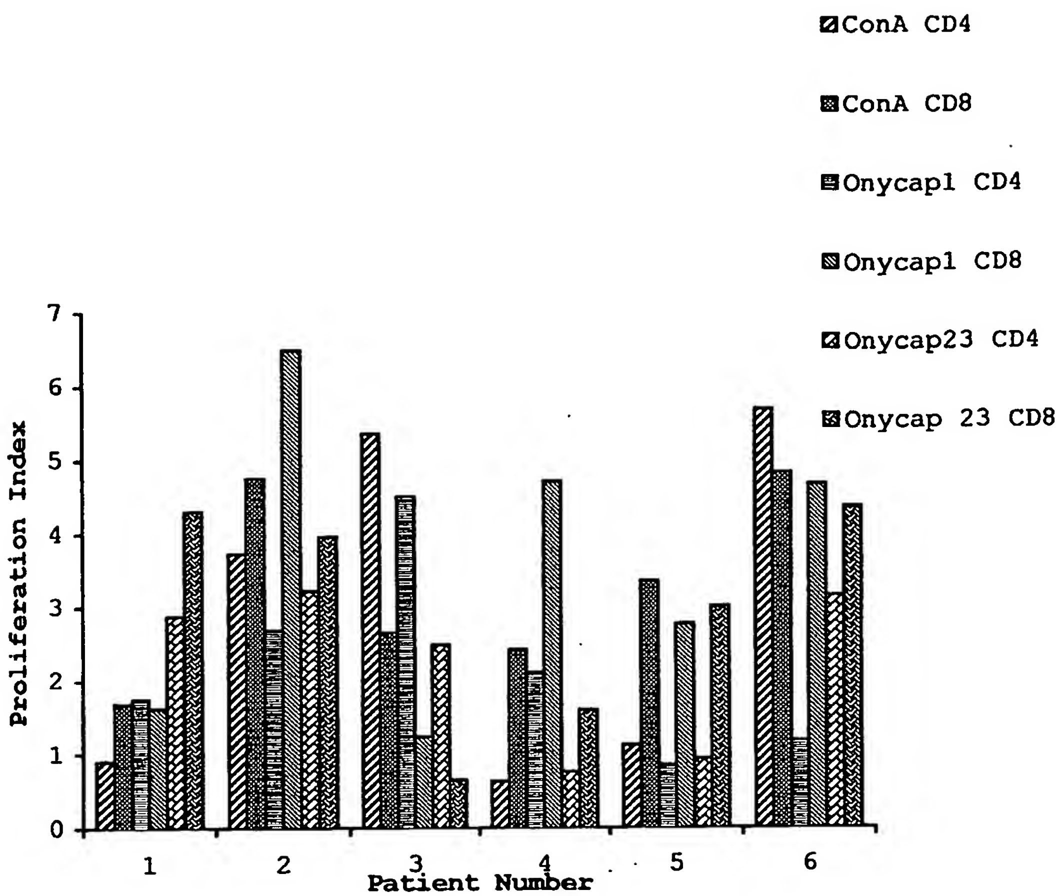
A = Isotype control plot
B = Specific antibody staining

Figure 3Biii



A = Isotype control plot
B = Specific antibody staining

Figure 4:


ConA CD4
ConA CD8
Onycap1 CD4
Onycap1 CD8
Onycap23 CD4
Onycap 23 CD8
Proliferation Index
0
1
2
3
4
5
6
7
1
2
3
4
5
6
Patient Number